CD36 Mediates the Phagocytosis of *Plasmodium falciparum*–Infected Erythrocytes by Rodent Macrophages

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Phagocytic cells represent an important line of innate defense against malaria; however, little is known of the mechanism by which macrophages recognize *Plasmodium falciparum*–parasitized erythrocytes (PEs). Using macrophages from CD36 wild-type (WT), CD36-null, and CD36 transgenically-rescued rodents, we demonstrate a major role for CD36 in the phagocytosis of PEs. WT macrophages display enhanced phagocytic capacity for nonopsonized PEs, compared with that for CD36-null mouse and rat macrophages. Transgenic rescue of CD36-deficient rats restored macrophage phagocytic capacity for PEs. CD36 receptor blockade with monoclonal antibodies and proteolytic cleavage of CD36 ligands from the surface of PEs inhibited the uptake of PEs. Up-regulation of rodent CD36 by use of peroxisome proliferator-activated receptor (PPARγ) agonists increased the phagocytosis of PEs. CD36-mediated uptake of PEs did not result in increased tumor necrosis factor–α secretion, of which high levels are associated with adverse outcomes in malaria. These studies support the use of these rodent models to examine PE-CD36 interactions.

Malaria caused by *Plasmodium falciparum* remains a major cause of global morbidity and mortality. Young children and other nonimmune individuals are at the greatest risk of developing severe and fatal malaria [1]. Adverse outcomes in falciparum malaria have been linked to an inability of the host to control parasite replication, which results in high parasite burdens, unbalanced or excessive secretion of proinflammatory cytokines, such as tumor necrosis factor (TNF)–α, in response to infection, and the sequestration of *P. falciparum*–parasitized erythrocytes (PEs) in the microvasculature of vital organs [1–5]. Several receptors have been implicated in the cytoadherence of PEs to endothelial cells, including intercellular adhesion molecule–1 (ICAM-1) [6], vascular cell adhesion molecule–1 (VCAM-1), E-selectin [7], platelet/endothelial cell adhesion molecule (PECAM)–1/CD31 [8], chondroitin 4-sulfate [9], thrombospondin [10], αvβ3 [11], and CD36 [12].

CD36 is a member of a novel family of scavenger receptors. It is found on endothelial cells and is believed to function as a sequestration receptor for malaria-infected erythrocytes, because it supports adherence of almost all natural isolates of *P. falciparum* [13, 14]. However, several lines of evidence question a direct role for CD36 in severe and cerebral malaria. Cerebral and glomerular microvascular endothelial cells have low or absent levels of CD36, and there is no evidence for increased endothelial expression of CD36 in severe malaria [4, 15]. Studies from areas where malaria is en-
demise have reported that higher levels of binding of PEs to CD36 are associated with nonsevere malaria [16, 17]. In addition, a population-based study by Aïtman et al. [18] reported that CD36-deficient individuals were at greater risk of developing severe and cerebral malaria. In contrast, expression of PE adhesion molecules, such as ICAM-1, is found in brain endothelium and has been correlated with cerebral sequestration of PEs [1, 4, 19]. Furthermore, expression of ICAM-1 is markedly up-regulated by proinflammatory cytokines, including TNF-α, excessive levels of which have been associated with adverse malaria outcomes [19–22].

Phagocytic cells, including monocytes and macrophages, are an essential first line of innate defense against malaria (reviewed in [23, 24]). During acute falciparum malaria in nonimmune individuals, it has been estimated that all erythrocytes should be infected within 10–12 days of liver schizont rupture. However, in actual clinical cases, the average parasitemia observed at this time is only 0.1%, which indicates that innate defense mechanisms, including the action of monocytes and macrophages, control parasite replication and invoke a 3–4-log reduction in parasite burden before antigen-specific immune responses develop [23]. However, the molecular basis by which macrophages recognize and clear PEs has not been fully elucidated. CD36 is found on monocytes and macrophages, and recent in vitro studies have demonstrated that this receptor mediates the phagocytosis of nonopsonized PEs by human monocytes and macrophages. Furthermore, peroxisome proliferator–activated receptor γ–retinoic X receptor (PPARγ-RXR) agonists, which up-regulate CD36 expression through direct promoter interactions, were shown to induce an increase in CD36-mediated phagocytosis and a decrease in malaria-induced TNF-α secretion by human monocytes and macrophages [25–27]. These data suggest that CD36-mediated nonopsonic phagocytosis of PEs may represent an important clearance pathway, particularly in nonimmune individuals in whom opsonic phagocytosis would be expected to be less than in semi-immune individuals. Furthermore, clinical outcomes in malaria may be improved by specific up-regulation of macrophage CD36. An animal model to evaluate the effects of pharmacologic modulation of CD36 would be of value in investigating the interaction of CD36 and malaria in vivo.

Several orthologues of human CD36 have been cloned and sequenced. Human, murine, and rat CD36 share ~90% sequence homology at the amino acid level, and rodent CD36 has been shown to avidly support the cytoadherence of PEs in vitro [28]. The objective of the present study was to investigate the role of CD36 in the uptake of nonopsonized P. falciparum–infected human erythrocytes by murine macrophages using CD36 wild-type (WT) and CD36-null macrophages generated by homologous recombination. These studies were extended by examining the uptake of PEs by macrophages isolated from spontaneous hypertensive rats (SHRs), which are deficient in CD36, and SHRs transgenically rescued for CD36 (SHR-Con4 and SHR-TG19). We also determined the effect of CD36 receptor modulation with PPARγ-RXR agonists on the expression of CD36 and subsequent PE phagocytosis. Here, we demonstrate that phagocytosis of nonopsonized PEs by rodent macrophages is mediated, in large part, by rodent CD36 and does not lead to increases in TNF-α and IL-6 secretion. Up-regulation of rodent CD36 using PPARγ-RXR agonists significantly increases the phagocytosis of PEs. Our data support the use of a rodent model for examining PE-CD36 interactions.

METHODS AND MATERIALS

Media and reagents. Endotoxin-free RPMI 1640 medium and heat-inactivated group AB human serum were purchased from Wisent. Monoclonal anti–mouse CD36 IgA (clone 63) was generated from hybridomas of spleen cells taken from CD36-null mice after intravenous injection of recombinant adenovirus containing full-length murine CD36 cDNA (the adenovirus was a gift from Fred deBeer, University of Kentucky). Anti–ICAM-1 clone 3E2, anti–α,β, clone R1-2, anti–CD45 clone 30-F11, anti–PECAM-1 clone MEC 13.3, and TNF-α and interleukin (IL)–6 ELISA sets were obtained from BD-Pharmingen. The anti–mouse CD19 clone MB19-1 was purchased from eBioscience. Mouse and rat Fc fragments were purchased from Jackson Immunochemicals. Troglitazone was purchased from BIOMOL. All other reagents were obtained from Sigma-Aldrich.

Parasite culture. Parasite cultures of the laboratory clone ITG were maintained by use of standard procedures, as described elsewhere [29]. Cultures were grown at a 5% hematocrit in 25-mL tissue culture flasks at 37° C in medium that consisted of RPMI 1640 supplemented with 6.0 g/L HEPES, 1.8 g/L NaHCO3, 1.35 mg/L hypoxanthine, and 10% heat-inactivated group AB serum. Erythrocytes were obtained as whole blood from healthy human volunteers. Cultures were gassed with a mixture of 3% CO2, 1% O2, and 96% N2. Twenty-four hours before phagocytosis assays were performed, cultures were synchronized by treatment with 5% sorbitol for 5 min at room temperature and were washed twice.

Isolation of peritoneal macrophages and phagocytosis assay. Resident peritoneal macrophages and thioglycollate-elicited macrophages (4 days after intraperitoneal injection of 2–4 mL of 4% sterile thioglycollate solution) were collected by peritoneal lavage into ice-cold PBS. Macrophages or RAW 264.4 cells plated at a density of 1.25 × 105 cells on autoclaved glass coverslips in 12-well polystyrene culture plates were cultured for 5 days in RPMI 1640 medium with 10% heat-inactivated fetal calf serum (FCS) and gentamicin (RPMI 10-G) at 37° C in 5% CO2 before use. Phagocytosis assays were performed, as described elsewhere [25]. To block Fc receptors, plated macro-
phages were pretreated with mouse or rat Fc fragments (20 μg/mL) for 20 min at 4°C. Macrophages then were exposed to highly synchronized schizont-stage PEs at a ratio of 20 PEs to 1 macrophage in 500 μL of RPMI 10-G, and were incubated for 4 h at 37°C in 5% CO₂, with gentle rocking. The proportion of uninfected erythrocytes was kept constant in these assays. Schizont-stage parasites were used because they uniformly express parasite-encoded ligands that interact with CD36. At the end of 4 h, coverslips were washed with ice-cold water for 45 s to lyse nonphagocytosed PEs and were fixed and stained with Diff-Quik. Phagocytosis was quantified by light microscopy, counting 500 macrophages/coverglass. Criteria for phagocytosis required the PE to be contained completely within the macrophage cell outline. Phagocytosis index was calculated as the percentage of macrophage with at least 1 internalized PE multiplied by the average number of PEs per macrophage for that coverslip [30].

Where indicated, monoclonal antibody (MAb) blockade was used to examine the contribution of various rodent macrophage surface receptors to the phagocytosis of PEs. Fc receptor–blocked macrophages were pretreated with the specified MAb for 20 min at 4°C and were washed twice with RPMI 1640 medium before exposure to PE. MAb against murine CD36, ICAM-1, CD49d, PECAM-1, CD45, and CD19 (isotype control) were used at 5 μg/mL.

To examine the role of PPARγ agonists, macrophages were treated with 50 μmol/L troglitazone and were incubated at 37°C in 5% CO₂ for 48 h. To remove P. falciparum erythrocyte membrane protein-1 (PfEMP-1), the parasite-encoded ligand for CD36, PEs were treated with a low concentration of trypsin (0.05%) for 30 min. PEs were washed twice with RPMI 1640 medium before incubating with macrophages.

**Macroage TNF-α and IL-6 secretion.** To determine TNF-α and IL-6 secretion due to macrophage-PE interactions, thioglycollate-elicited and resident peritoneal murine macrophages were plated, as described above. Four hours after exposure to washed and synchronized PEs (20:1), or lipopolysaccharide (LPS; 1 μg/mL), or uninfected erythrocytes (UEs), cell-free supernatants were collected and assayed for soluble TNF-α and IL-6 by use of a sandwich ELISA, according to the manufacturer’s instructions.

**Flow cytometry.** To determine macrophage surface levels of CD36, murine and rat macrophages were stained with anti–CD36 MAb (1:100) for 30 min and then with a secondary anti-mouse IgA–fluorescein isothiocyanate (FITC) conjugate antibody (1:16) for 30 min on ice. Controls were stained with the secondary antibody alone. Monocytes then were fixed with 0.5% paraformaldehyde/PBS and were analyzed by use of EPICS ELITE flow cytometer and software (Beckman-Coulter).

**Statistical analysis.** All experiments were performed in duplicate or triplicate and were repeated at least 3 times. Data are mean ± SEM, unless otherwise noted. Statistical significance was determined by use of the Student’s t test.

**RESULTS**

**Murine CD36 mediates the uptake of nonopsonized PEs.** Previous in vitro studies have implicated human CD36 (hCD36) in the uptake of nonopsonized PEs by human macrophages; however, these studies were limited by the lack of macrophages null for CD36 [25, 26]. In the present study, we investigated the ability of murine macrophages to participate in nonopsonic PE phagocytosis and determined whether murine CD36 contributed to this process. We examined the uptake of PEs by murine CD36 WT murine macrophages and by murine macrophages rendered murine CD36 null (KO) by targeted homologous recombination in C57BL/6 mice (figure 1). There was an ~85% decrease in the uptake of nonopsonized PEs by thioglycollate-elicited CD36-KO macrophages, compared with that in CD36 WT macrophages. Phagocytosis occurred in the absence of complement, with Fc receptor blockade, and without prior opsonization of PEs. MAb blockade of CD36 resulted in an 80% decrease in the uptake of nonopsonized PEs by WT macrophages but did not significantly affect the low uptake of PEs by KO macrophages (figure 1). Experiments that used resident peritoneal macrophages from WT and KO mice demonstrated similar results, with resident KO macrophages displaying significantly lower uptake of PEs versus WT resident macrophages (phagocytic index: WT, 41.35 ± 4.39; KO, 20.45 ± 6.7; P < .01). Phagocytosis of uninfected erythrocytes by murine macrophages was not observed.

PfEMP-1, a parasite-encoded ligand, has been shown to mediate the binding of PEs to human CD36 [31]. We wished to determine whether PfEMP-1 or other trypsin-sensitive ligands mediated the binding of PEs to murine CD36. Removal of the CD36 ligand from PEs by mild trypsinization resulted in a decrease in phagocytosis similar to that observed with murine CD36 KO macrophages (figure 2). Antibody blockade of other receptors and an IgA isotype control MAb in a significant inhibition of nonopsonic PE uptake to the level observed with CD36 KO macrophages (figure 2). Antibody blockade of other receptors and an IgA isotype control MAb (CD19) alone and in combination did not significantly reduce the uptake of PEs by WT or KO macrophages (figure 2). These results support a role for murine CD36 as the major receptor.
Figure 1. Murine CD36 mediates the phagocytosis of nonopsonized Plasmodium falciparum–parasitized erythrocytes (PEs) by murine macrophages. A, CD36 mean log10 fluorescence of CD36 wild-type (WT) macrophages (dark gray) and CD36-null (KO) macrophages (light gray) is shown. Secondary-only stained control is shown as a black dashed line. Flow cytometry histograms shown are typical of independent experiments. B, Thioglycollate-elicited CD36 WT and KO macrophages adherent to glass coverslips were pretreated with Fc fragments and were incubated with cultures of nonopsonized PEs for 4 h. The diagonal hatched bar represents macrophages pretreated with anti–CD36 antibody (5 μg/mL). The horizontal hatched bar represents trypsin treatment of PEs before a phagocytosis assay was performed. Phagocytosis assays were performed, as described in Material and Methods. Experiments were performed in duplicate or triplicate and repeated at least 3 times. **P < .01, CD36-WT control vs. CD36-KO control; ***P < .01 CD36-WT control vs. CD36-KO; and CD36-WT control vs. trypsinized PEs.

mediating the phagocytosis of nonopsonized PEs by murine macrophages in vitro.

We sought to determine whether these findings were generalizable to other inbred lines of mice and to murine cell lines. We examined PE uptake by thioglycollate-elicited macrophage isolated from BALB/c mice and by the transformed murine monocyte cell line RAW 264.4. Macrophages from BALB/c and RAW 264.4 cells were able to phagocytose PEs in the absence of complement or PE opsonization (phagocytic index: BALB/c, 22.17% ± 7.64; RAW 264.4, 14.98 ± 1.46). By use of various MAbs, as described above, only CD36 receptor blockade or cleavage of CD36 ligands by trypsin treatment of PEs significantly decreased the uptake of PEs (data not shown).

Up-regulation of murine macrophage CD36 with PPARγ-RXR agonists increases their phagocytic capacity for PEs. Previous studies have shown that human monocytes and cultured-derived macrophages treated with PPARγ-RXR agonists increase their surface levels of CD36 and their phagocytic capacity for nonopsonized PEs [26, 27]. On the basis of these studies, we hypothesized that an increase in murine CD36 surface levels on murine macrophages would lead to an increase in phagocytosis of nonopsonized PEs. Results from flow cytometry analysis showed that murine macrophages treated with troglitazone for 48 h showed significantly higher surface levels of CD36 (figure 3A). In addition, troglitazone-treated macrophages from C57BL/6, BALB/c, and RAW 264.4 displayed significantly increased phagocytic capacity for PEs, compared with untreated macrophages (figure 3B). In each case, this increase in phagocytosis was inhibited with anti–CD36 MAb (data not shown). These data support the hypothesis that therapeutic agents that increase surface levels of CD36 may enhance the phagocytic clearance of nonopsonized PEs.

Nonopsonic uptake of PEs by murine macrophages does not induce TNF-α or IL-6 secretion. Elevated levels of proinflammatory cytokines, including TNF-α and IL-6, in response to malaria infection are associated with adverse outcomes in human malaria and in murine malaria models. To determine whether murine CD36-mediated adherence and uptake induced TNF-α and IL-6 secretion, we examined murine CD36 WT, CD36 KO, BALB/c, and RAW macrophages for cytokine secretion during PE phagocytosis assays. Nonopsonic uptake of tightly synchronized and well-washed trophozoite-stage PEs by these cells did not induce secretion of TNF-α or IL-6 (figure 4A and 4B).

Transgenic rescue of CD36 deficiency in rat macrophages restores their phagocytic capacity for nonopsonized PEs. SHRs, a model for insulin-resistant diabetes, have been noted to carry mutations in their CD36 locus, rendering them deficient for functional CD36 [32, 33]. Kurtz et al. [34] recently transgenically (SHR-TG19) and congenically (SHR-Con4) rescued CD36 function in the SHR line. To confirm and extend
Figure 2. Murine CD36 is a primary receptor involved in the phagocytosis of nonopsonized *Plasmodium falciparum*-parasitized erythrocytes (PEs). Prior to phagocytosis assay, CD36–wild-type (WT) and –null (KO) monocytes blocked with Fc fragments were pretreated, either alone or in combination, with monoclonal antibodies (5 μg/mL) against various murine surface receptors, including CD36, CD54 (intercellular adhesion molecule–1), CD49d (α4), CD45 (leukocyte common antigen), and CD31 (platelet/endothelial cell adhesion molecular–1). Experiments were performed in duplicate or triplicate and were repeated at least 3 times. **P < .01, CD36-WT control vs. CD36-WT CD36 blockade; ***P < .01, CD36-WT control vs. CD36-KO control.

![Phagocytic Index (%)](image)

CD36-WT
CD36-KO
anti-CD36
anti-CD54
anti-CD49
anti-CD45
anti-CD31

Our studies of the role of CD36 in PE uptake, we compared nonopsonic PE phagocytosis in macrophages derived from Wistar Kyoto (WK) rats (rat CD36 WT), SHRs (rat CD36 deficient), SHRs-TG19 (CD36 deficiency transgenically rescued), and SHRs-Con4 (CD36 deficiency congenically rescued). Using flow cytometry, we confirmed that WK macrophages expressed high levels of CD36, whereas SHR macrophages were deficient for surface CD36. SHR-Con4 and SHR-TG19 macrophages were surface positive for CD36 but at lower levels than WK macrophages (figure 5A). Corresponding to the presence of cell surface CD36, macrophages from WK rats, SHRs-Con4, and SHRs-TG19 internalized significantly higher numbers of nonopsonized PEs than did macrophages from SHRs (figure 5B). Macrophages from SHRs-Con4 and SHRs-TG19 internalized more PEs than SHR macrophages but less than WK macrophages, which correlates with their lower surface levels of CD36. Pretreatment of macrophages with anti–CD36 MAb inhibited the phagocytosis of PEs by macrophages from WK rats, SHRs-Con4, and SHRs-TG19 to the levels observed with SHR macrophages. Furthermore, cleavage of trypsin-sensitive ligands on surface of PEs significantly reduced the uptake of PEs by macrophages from WK rats, SHRs-Con4, and SHRs-TG19. These data using transgenomic rescue of CD36 confirm that it is a primary receptor involved in the uptake of nonopsonized PEs by rodent macrophages in vitro.

**DISCUSSION**

In the present study, we have demonstrated that rodent macrophages are capable of internalizing nonopsonized PEs. Using CD36 expressing (WT) and CD36-null (KO) murine macrophages, we have shown that CD36 is the major receptor involved in this phagocytic process in vitro. CD36 KO macrophages showed a significant reduction in phagocytosis of PEs, compared with that of CD36 WT macrophages. Uptake of PEs by WT macrophages was blocked (>80%) by anti–murine CD36 antibodies and by cleavage of the PE ligand for CD36 (>80%), but not by receptor blockade of other murine macrophage receptors, including ICAM-1, α4, CD45, and PECAM-1. Similar results were observed when primary macrophages from BALB/c and murine-transformed cell line RAW cells were used in PE phagocytosis assays. We have also shown that pretreatment of macrophages with PPARγ agonists, which increase the expression of CD36, significantly increases the phagocytosis of PEs. Finally, using rat macrophages that either express normal levels of CD36, are CD36 deficient, or have been transgenically...
rescued for CD36, we have demonstrated that CD36 is a primary receptor involved in the uptake of nonopsonized PEs. The use of CD36-null macrophages, as well as transgenically rescued macrophages, provides the strongest evidence, to our knowledge, for a central role for CD36 in the nonopsonic uptake of PEs. The present study confirms and extends previous studies examining the role of human CD36 in the uptake of asexual stage *P. falciparum* by human monocytes/macrophages [25–27].

The molecular mechanisms underlying severe and cerebral malaria are not completely understood. Adverse outcomes have
Figure 4. Uptake of *Plasmodium falciparum*–parasitized erythrocytes (PEs) by murine macrophages is a noninflammatory process. Thiglycollate-induced macrophages from C57BL/6 wild-type (WT) (panel 1, light gray) and –null (KO) mice (panel 1, dark gray), BALB/c (panel 2), and RAW 264.4 (panel 3) were incubated with PEs, uninfected erythrocytes (UEs), or lipopolysaccharide (LPS) (1 μg/mL) for 4 h. Cell-free supernatants were collected and analyzed for tumor necrosis factor (TNF)-α (A) and interleukin (IL)-6 (B) secretion by ELISA. Data are representative of results collected from 4 independent experiments.

been linked, in part, to unbalanced or excessive levels of pro-inflammatory cytokines, including TNF-α and IL-6. In the present study, we have demonstrated that phagocytosis of PEs by murine macrophages does not result in a significant increase in TNF-α or IL-6 secretion, which is consistent with previous observations with human monocytes [25, 26]. In contrast, previous studies reported an association between uptake of PEs and TNF-α secretion [35, 36]. However, these previous studies
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Figure 5. Transgenic rescue of CD36 in spontaneous hypertensive rats (SHRs) restores the phagocytic capacity of macrophages for *Plasmodium falciparum*-parasitized erythrocytes (PEs). We also performed phagocytosis assays with CD36 wild-type rat macrophages (Wistar), CD36-deficient macrophages (SHR), or SHR macrophages that had been transgenically (TG19) or congenically (Con4) rescued for CD36, to further support the role of CD36 phagocytosis of nonopsonized PEs. Thioglycollate-induced macrophages were isolated and plated, as described in Methods and Materials. A, Macrophages isolated from rats were stained for CD36 surface levels and are shown as mean fluorescence over background. B, Macrophages adherent to glass coverslips were pretreated with Fc fragments to block Fc receptors and then were incubated with cultures of nonopsonized PEs for 4 h. Diagonal hatched bars indicate that Fc blocked macrophages that were pretreated with anti–CD36 monoclonal antibody. Horizontal hatched bars indicate trypsin treatment of PEs before phagocytosis assays. Experiments were performed in duplicate or triplicate and were repeated at least 3 times. **, PE control vs. CD36 blockade and PE control vs. trypsin treatment; xx, Wistar control vs. SHR control; §, SHR control vs. Con4 control and SHR control vs. TG19 control.

were performed with less tightly–synchronized parasite cultures, and the observed TNF-α secretion may reflect the presence of contaminating parasite-derived glycosylphosphatidylinositol (GPI) toxins known to induce proinflammatory cytokines [37]. Alternatively, TNF-α secretion may have been due to the presence of opsonizing PE antibodies allowing for Fc receptor–mediated phagocytosis to occur [38, 39]. These potential confounders were not present in the present study, because we used tightly synchronized and well-washed PEs to minimize GPI contamination, and our phagocytosis assays were performed with nonopsonized PEs and with murine macrophages that were pretreated with Fc-fragments to block Fc receptors.

In the present study, troglitazone, a PPARγ agonist, increased CD36 expression in murine macrophages, which was associated with an increase in uptake of PEs. Previously, PPARγ-RXR agonists were reported to increase CD36 expression and PE uptake and to decrease TNF-α secretion in response to GPI toxins in human monocytes/macrophages [25–27]. PPARγ agonists have been demonstrated to influence a variety of cellular processes, including modifying proinflammatory cytokine responses via both PPARγ-dependent and -independent pathways [40]. Therefore, some of the observed effects on PE uptake and cytokine secretion induced by troglitazone could be mediated via CD36- and PPARγ-independent mechanisms.

The interaction of CD36 and *falciparum* malaria in vivo is undoubtedly complex. Most natural *falciparum* isolates avidly bind to CD36, even under conditions of shear stress, which supports its role as a sequestration receptor. Because CD36 expression is low to absent in endothelia of brain and glomeruli but is highly expressed in liver, spleen, muscle, adipose tissue, and skin microvascular vessels, CD36-mediated sequestration probably predominates in these latter tissues [4]. Several studies have reported that the CD36–PE interaction is associated with nonsevere malaria [16, 17], and Aitman et al. [18] have demonstrated a significant association between CD36 deficiency and increased susceptibility to severe and, especially, cerebral malaria. It has been suggested that CD36-mediated sequestration in nonvital sites may be protective [18, 26]. Although complex, our data also would appear to support, at least in part, a potentially beneficial role for CD36, particularly in nonimmune individuals, by facilitating clearance of PEs in a noninflammatory manner and thus lowering parasite burdens and decreasing cytokine-mediated up-regulation of brain endothelial cell adhesion molecules. Furthermore, these in vitro studies suggest that the CD36-mediated clearance of PEs and inflammatory responses to infection can be beneficially modulated using PPARγ-RXR agonists. On the other hand, adhesion and uptake of PEs and hemozoin have been reported to inhibit macrophage and dendritic cell maturation and/or function (reviewed in [41]). An appropriate model with which to clarify the interaction of PEs with CD36 in vivo would represent an important advance. The use of mouse cells and murine models to study *P. falciparum* clearance offers several advantages over primate models or human clinical trials, including the avail-
ability of gene knockouts, congenic lines, and well-characterized reagents to evaluate immune responses and signal transduction pathways.

The present study has demonstrated that rodent macrophages can be used as an in vitro model to study the binding and uptake of *P. falciparum*-infected erythrocytes. It further suggests that the CD36 KO model may provide an opportunity to examine the role of murine CD36-mediated clearance and sequestration in vivo, including the effects of CD36 receptor modulation in vivo with PPAR-α/RXR ligands. However, these studies may be restricted to examining the phagocytic capacity of peritoneal macrophages in vivo after intraperitoneal injection of PEs. Whether the effects of CD36 receptor modulation in vivo also can be assessed in these models by use of rodent *Plasmodium chabaudi* malaria, which can cytoadhere and may bind murine CD36 [42], is currently under investigation.

References


